

Patent claims

1. Biologically pure bacterial culture of at least one mutant strain of *P. fluorescens*, wherein said strain produces at least 10 g alginate per liter medium.
2. Biologically pure bacterial culture of at least one mutant strain of *P. fluorescens* of claim 1, wherein said strain produces at least 10 g alginate per 40-55 g carbon source per liter medium.
3. Biologically pure bacterial culture of at least one mutant strain of *P. fluorescens* of claim 1, wherein said strain produces at least 10 g alginate per 50-55 g carbon source per liter medium.
4. Biologically pure bacterial culture of at least one mutant strain of *P. fluorescens* of claim 1, wherein said strain produces at least 10 g alginate per 40 g carbon source per liter medium.
5. Pure mutant strain of *P. fluorescens* of claim 1, wherein said mutant strain is selected from the group consisting of the mutant strain Pf201, Pf2012, Pf2013, Pf20118, Pf20137, Pf20118algIΔ, Pf 20118algFΔ, Pf20118AlgLH203R and Pf201MC.
6. Pure mutant strain of *P. fluorescens* of the claims 1, wherein the said mutant is capable of producing large amounts of an alginate consisting of manuronate residues only.
7. Pure mutant strain of *P. fluorescens* of the claim 5 and 6, wherein the said mutant is selected from the group consisting of the variant strains Pf2012, Pf2013, Pf20118, and Pf20137.
8. Pure mutant strain of *P. fluorescens* of claim 1, wherein the said mutant is capable of producing alginate having a defined guluronate residue (G)-content between 0 and 30 %.

9. Pure mutant strain of *P. fluorescens* of the claims 1, wherein the said mutant is capable of producing alginate without, or with a reduced number of O-acetyl groups.

10. Pure mutant strain of *P. fluorescens* of the claim 5 and 9, wherein the said mutant is selected from the group consisting of the mutant variant strains Pf20118algIJΔ and Pf 20118algFΔ.

11. Pure mutant strain of *P. fluorescens* of claim 1, 2, 3, 4 or 5 wherein the said mutant is capable of producing alginate with a molecular weight of between 50,000 and 3,000,000 Daltons.

12. Pure mutant strain of *P. fluorescens* of claim 11, wherein the said mutant is selected from the group of the variant mutant strain Pf20118AlgLH203R.

13. Pure mutant strain of *P. fluorescens* of claim 1, 2, 3, 4 or 5 comprising an alginate biosynthetic operon regulated by an inducible promoter different from the naturally occurring promoter, and optionally one or more effector genes.

14. Pure mutant strain of *P. fluorescens* of claim 13, wherein the inducible promoter is a *Pm* promoter, and further comprising the effector gene *xyIS*.

15. Pure mutant strain of *P. fluorescens* of claim 13 and 14, wherein the said mutant strain is Pf201MC.

16. Method of producing the novel mutant strain of *P. fluorescens* of claim 1, wherein :

- (a) a wild-type strain of *P. fluorescens* is contacted with a mutagenic agent, and
- (b) the treated bacteria of step (a) are grown in the presence of one or more antibiotics, and
- (c) antibiotic resistant mucoid mutants are isolated by selection, and
- (d) the alginate production properties of the isolated mucoid mutants of step (c) are determined.

17. Method according to claim 16, wherein the mutagenic agent is nitrosoguanidine.

18. Method according to the claims 16 or 17, wherein the treated bacteria of step (a) are grown in the presence of a β -lactam or aminoglycoside antibiotic.

19. Method according to claims 16 or 17 wherein the treated bacteria of step (a) are grown in the presence of carbenicillin.

20. Method of producing a mutant strain of *P. fluorescens* of claim 13, wherein
(i) the alginate biosynthetic operon promoter of a wild type strain of *P. fluorescens* is exchanged by an inducible promoter by homologous recombination, and
(ii) optional effector genes are introduced into the bacterium of (i) by homologous recombination, transposon mutagenesis or by means of a plasmid, and
(iii) mutants are grown and then isolated by selection, and
(iv) the alginate production properties of the isolated mutants of (iii) are determined.

21. Method according to claim 20, wherein the inducible promoter is *Pm* from *P. putida* Tol-plasmid, or a mutated *Pm* promoter.

22. Method of producing a mutant strain of *P. fluorescens* of claim 8, characterized in that

- a) the wild type *algG*-gene, encoding the C-5 epimerase is cloned in a plasmid or minitransposon and mutagenized by chemical mutagenesis or by PCR,
- b) a derivative of an alginate-producing strain of *P. fluorescens*, which lacks the *algG* gene (Δ *algG*-strain), is constructed, and
- c) the library of mutagenized *algG* of step (a) is transferred to the Δ *algG*-strain of *P. fluorescens*, and the plasmid or transposon-containing strains were identified and assayed for alginate-production and epimerase-activity, and
- d) the plasmid or transposon-containing strains containing a mutant *algG* encoding an epimerase that provides alginate with a guluronic acid residue content between 0 and 30 % are identified by the assay in step (c), and
- e) the mutant *algG* gene is cloned into a gene-replacement vector, and

f) the gene-replacement vector of step (e) is then transferred to an alginate-producing strain of *P. fluorescens* in order to replace its *algG* gene with the mutant *algG* gene, and making it capable of expressing the mutant gene.

23. Method of producing a mutant strain of *P. fluorescens* of claim 8, characterized in that

- a) one or more amino acids, which is identified by mutagenesis and subsequent screening to be important for epimerization, is exchanged, at the gene-level, by site-specific mutagenesis to amino acids different from the ones occurring both in the mutant and the wild-type AlgG-protein, and
- b) the mutant gene is cloned into a gene-replacement vector and this vector is transferred to an alginate-producing strain of *P. fluorescens* where it replaces the wild-type *algG* gene and is capable of being expressed.

24. Use of biologically pure bacterial culture of at least one mutant strain of *P. fluorescens* of any of the previous claims 1-15, for the production of alginate.

25. Use of biologically pure bacterial culture of at least one mutant strain of *P. fluorescens* of any of the previous claims 1-15, for the large scale fermentor-production of alginate.

26. Use of the alginate produced by at least one mutant strain of *P. fluorescens* of any of the previous claims 1-15, in the preparation of a food or industrial product such as a pharmaceutical, cosmetic, animal feed or nutrient product, or as an intermediate product for *in vitro* C-5-epimerization.